REMARKS

Reconsideration of the rejections set forth in the Office action mailed May 15, 2002 is respectfully requested. Claims 1-20 are currently under examination.

I. Amendments

Claims 1 and 10 have been amended to specify that the antisense oligomer is a morpholino oligomer, having a substantially uncharged backbone, and that the target sequence spans the translational start codon or an intron or exon junction site of the target mRNA. Claim 17 is also amended to specify that the antisense oligomer is a morpholino oligomer. Support is found in the specification at, for example, page 22, line 7, as well as the discussion of morpholino oligomers at page 7; in original claims 4 and 13 (substantially uncharged backbone); and at page 15, lines 34-37 (target regions).

Claims 5 and 14 have been amended to specify an embodiment in which the morpholino subunits are linked by phosphorodiamidate intersubunit linkages, joining a morpholino nitrogen of one morpholino subunit to a 5'-exocyclic carbon of an adjacent morpholino subunit. Support is found, for example, at page 7, lines 22-29.

Claim 18 has been amended to recite specific base sequences of the morpholino oligomers. Support is found, for example, at page 4, lines 30-32.

Claim 19 has been redrafted in independent form, incorporating the limitations of the parent claims (in their form prior to the present amendments).

Support for the amendment to claim 20 is found, for example, at page 22, lines 23-20 (pharmaceutical carrier).

Several claims (3-4, 6-8, 12-13, 15-16, and 18) have been amended solely for clarity, generally for grammatical correctness.

No new matter is added by any of the amendments.

II. Allowable Subject Matter

Claim 19 was found allowable but objected to as being dependent on a rejected base claim. As noted above, claim 19 has been redrafted in independent form.

III. Rejections under 35 U.S.C. §112, First Paragraph

Claims 1-16 and 20 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and use the invention without undue experimentation. The Examiner contends that the disclosure does not enable the full scope of the claimed invention.

Applicants note that, by the present amendments, independent claims 1, 10 and 17 (on which claim 20 is dependent) specify that the antisense oligomers are morpholino oligomers, as described in the specification at, for example, page 7, and that the target sequence spans a start site or splice junction of the target mRNA.

The invention of claim 1 is directed to a method of modulating hematopoietic stem cell differentiation, by contacting such cells with one or more antisense morpholino oligomers, each having a substantially uncharged backbone and a base sequence directed to a target sequence spanning the translational start codon or an intron or exon junction site of an mRNA preferentially expressed in stem cells. In accordance with claim 1, such contacting is effective to achieve (i) an increase in the number of lineage committed progenitor cells and their progeny, and/or (ii) a slowing or diminution of the growth of cells exhibiting a loss of growth control, or a reduction in the total number of such cells. In accordance with claim 10, discussed further below, such cells are obtained from a subject and later restored to the subject after antisense oligomer treatment.

Example 1 of the specification shows how one may identify mRNA's which are "preferentially expressed in stem cells", using differential expression analysis as is known in the art. Example 2 shows that an antisense morpholino oligomer (SEQ ID NO: 1) directed to the translation start region of an mRNA preferentially expressed in stem cells (specifically, EVI-1 zinc finger mRNA) was effective to decrease the number of high proliferative potential colony forming cells (HPP-CFC), indicating modulation of the stem cell differentiation, in a dose dependent manner. A sequence-scrambled control oligomer was ineffective.

The Examiner contends that the disclosure of the specification does not enable *in vitro*, *in vivo* and *ex vivo* use of the oligomers as recited in claims 1 and 10. In support of the rejection, in the Office Action mailed July 31, 2001, the Examiner referred, at pages 4-5, to two articles (Branch and Crooke) which describe various difficulties encountered in antisense technology, including inconsistent uptake, nonspecific effects, toxicity, etc. However, these articles are

directed almost exclusively to negatively charged oligonucleotides, particularly phosphorothioate oligonucleotides. Branch (*TIBS* **23**:45-50, 1998), an oft-cited article discussing problems in antisense technology, particularly non-specific effects (Abstract), shows a predominating focus on phosphorothioates and other highly charged oligomers. Abstracts of several references cited in the article, where the subject oligomers are not clear from the article itself, show that the cited studies are all concerned with phosphorothioate and/or phosphodiester (native DNA) oligonucleotides (e.g. refs. 8, 17-19, 25-27, and 35). The chapter by Crooke (in *Antisense Research and Application*, Crooke, Ed., Springer-Verlag, 1998), including the section at pages 34-36 particularly pointed out by the Examiner, also focuses largely on phosphorothioate oligonucleotides.

As discussed in the enclosed Declaration under 37 CFR §1.132, the use of uncharged or substantially uncharged morpholino oligomers in antisense applications has been shown to overcome many of the drawbacks which are associated with charged antisense oligonucleotide analogs, such as the widely used phosphorothioate-linked oligonucleotide analogs. Nonspecific effects observed with charged, RNAse-competent oligomers, such as the phosphorothioates, are generally attributed to nonspecific binding, both to nontargeted nucleic acids and to cellular proteins, and nonspecific RNAse activation (which cleaves nontarget RNA, as shown in Fig. 1 of Branch). These effects are greatly reduced by the use of morpholino oligomers, in large part due to their minimal charge, or lack of charge, and mechanism of action, which is based on steric blocking rather than cleavage of the target.

Cell uptake and nuclease resistance of substantially uncharged morpholino oligomers has been demonstrated by experiments in which the oligomer is administered to a mammal and can be detected several hours later in body fluids, in the form of a duplex with the target mRNA (PCT Pubn. Nos. WO 2000/45167; WO 2002/48405). This shows that the oligomer enters cells and binds with target mRNA, and that the resulting duplex is resistant to nuclease digestion.

As discussed in the Declaration and appended references, morpholino oligomers having substantially uncharged, phosphorus-based linkages have shown sequence-specific antisense activity *in vivo* in a variety of animal models and targets.

One of the factors to be considered in determining whether a disclosure would require undue experimentation is "the nature of the invention" (*In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed.

Cir. 1988). Claims 1-16 are directed, not to phosphorothioate or other non-morpholino antisense oligomers, but to the use of morpholino oligomers having substantially uncharged backbone linkages. In view of the advancements provided by morpholino antisense technology, as pointed out in the enclosed Declaration, discussions of the state of the art in (primarily) phosphorothioate antisense technology are not effective as evidence of nonenablement of these claims.

With further reference to claim 10, the Examiner also cited, in the Office Action mailed July 31, 2001, a review article on gene therapy by Palu *et al.* (*J. Biotech.* **68**:1-13, 1998). The Examiner stated, with reference to the article, that "gene delivery using various vectors is dependent on empirical determination of successful gene transduction for a given vector and target cell" (Office Action, page 5). However, the applicants' claim 20 does not involve gene transduction, i.e., the transfer of genetic material into a cell via a vector, such that the material is incorporated into the genome of the cell. Rather, the claim involves contacting cells with the antisense oligomers of the invention, to modulate hematopoietic stem cell differentiation. As discussed above, the antisense oligomers of the invention are effective to enter cells (without the use of a vector) and inhibit translation of a target RNA. Difficulties in transduction of cells, as discussed by Palu, are not pertinent to this claim.

With further reference to claim 20, this claim is directed to a composition comprising an antisense oligomer having a substantially uncharged backbone, and further characterized by (a) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C, (b) nuclease resistance, and (c) the capability for active or facilitated transport into cells, and having the sequence presented as SEQ ID NO:1, where the composition further comprises a pharmaceutical carrier. It would be well within the ability of one of ordinary skill in the art to make and use this composition, based on the specification (e.g. Examples 1-2 above) and knowledge in the art.

In view of the foregoing, the applicants respectfully request that the rejections under 35 U.S.C. §112, first paragraph be withdrawn.

IV. Rejections under 35 U.S.C. §112, Second Paragraph

Claims 5 and 14 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite

for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the claims were objected to as referred to drawings in the specification. The claims have been amended to remove the reference to the figures, referring to the structural embodiment by name.

In view of the foregoing, the applicants submit that the amended claims comply with the requirements of 35 U.S.C. §112, second paragraph.

V. Rejections under 35 U.S.C. §103

Claims 17-18 were rejected under 35 U.S.C. §103 as being unpatentable over Mitani *et al.* (*Blood* **84**(10, Suppl 1):229a, 1994 and/or Molecular Biology of Hematopoiesis, 8th Symposium, July 9-13, 1993, Basel, Switzerland, p. 79) in view of Baracchini *et al.* (U.S. Patent No. 5,801,154). The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The applicant's invention, as embodied in claim 17, is directed to a composition comprising an antisense morpholino oligomer characterized by a backbone which is substantially uncharged, where the oligomer is directed to a sequence spanning the mRNA translational start codon of a gene preferentially expressed in stem cells. Claim 18, as amended, is directed to oligomers having selected base sequences.

B. The Cited Art

The Mitani abstracts discuss the investigation of a fusion protein (AML1/EVI-1) generated by a chromosomal abnormality found in blastic crisis of CML (chronic myelocytic leukemia), and antisense oligomers targeting the chimeric gene encoding the fusion protein. There is no suggestion in the reference to produce antisense oligomers directed to "mRNA preferentially expressed in stem cells".

In addition, as noted in the previous response (filed January 31, 2002), the Mitani abstracts give no information as to the structural nature of the synthetic antisense oligonucleotides employed.

As also noted in the previous response, Baracchini *et al.* describe a large number of possible modifications that could be used in preparing an antisense oligomer (column 6 of patent). However, the oligomers exemplified in the patent (see Tables 1-4) include only oligomers having

fully charged backbones; that is, phosphorothioate oligomers and phosphorothioate-phosphodiester mixed-backbone oligomers.

C. Analysis

The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that the invention should be carried out and would have a reasonable likelihood of success, viewed in light of the prior art. Both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure. *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988). That a prior art device could be modified to produce the claimed device does not justify an obviousness rejection unless the prior art suggested the modification's desirability. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

It is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. *Bausch & Lomb v. Barnes-Hind/Hydrocurve* (796 F.2d 443, 230 USPQ 416, Fed. Cir. 1986)

In view of the large number of possible structural types disclosed in Baracchini, the emphasis therein on charged-backbone oligonucleotide analogs, and the absence of teaching in Mitani as to backbone structure, the cited art provides no suggestion of the desirability of using a substantially uncharged morpholino oligomer, as recited in claim 19; nor is there any suggestion of the benefits provided by such use.

Nor is there any suggestion in either reference to produce antisense oligomers targeted to "mRNA preferentially expressed in stem cells". In particular, there is no suggestion to provide the specific-sequence morpholino oligomers of claim 18.

Accordingly, the applicants respectfully request the Examiner to withdraw the rejections under 35 U.S.C. §103(a).

VI. Conclusion

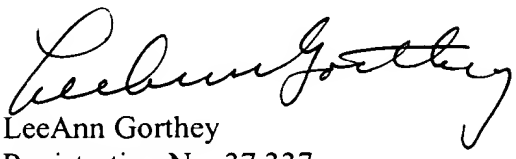
In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

No further fees are believed due with this communication. However, the Commissioner is

hereby authorized and requested to charge any deficiency in fees herein to Deposit Account No. 50-2207.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4403.

Respectfully submitted,



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Amendments to claims filed October 15, 2002
U.S. Serial No. 09/684,061

1. (Twice Amended) A method of modulating hematopoietic stem cell differentiation, comprising:

[providing] contacting said cells with one or more antisense morpholino oligomers, having a substantially uncharged backbone and a base sequence directed to a target sequence spanning the translational start codon or an intron or exon junction site of an mRNA preferentially expressed in stem cells,

wherein said contacting is effective to achieve (i) an [(i)] increase in the number of lineage committed progenitor cells and their progeny, and/or (ii) [effect] a slowing or diminution of the growth of cells exhibiting a loss of growth control, or a reduction in the total number of such cells.

3. (Amended) The method of claim 1, wherein each of said one or more antisense oligomers has a length of about 12 to 25 bases.

4. (Amended) The method of claim 1, wherein each of said one or more antisense oligomers is characterized by[,]

(a) a backbone which is substantially uncharged;

(b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;

(c) nuclease resistance; and

(d) the capability for active or facilitated transport into cells.

5. (Amended) The method of claim 1, wherein said antisense morpholino oligomer [backbone has a structure selected from the group consisting of the structures presented in Figures 2 A-A through 2 E-E] comprises phosphorodiamidate intersubunit linkages, joining a morpholino nitrogen of one morpholino subunit to a 5'-exocyclic carbon of an adjacent morpholino subunit.

6. (Amended) The method according to claim 2, wherein each of said one or more antisense oligomers has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ

ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.

7. (Amended) The method according to claim 6, wherein each of said one or more antisense oligomers has the sequence presented as SEQ ID NO:1.

8. (Twice Amended) The method according to any one of claims 1 to 7, wherein said one or more antisense oligomers [is] are provided to a subject in an amount sufficient to result in a peak blood concentration of at least 200-400 nM.

10. (Twice Amended) A method of modulating hematopoietic stem cell differentiation, comprising:

(a) obtaining a stem cell-containing cell population from a subject;

(b) treating the cell population in manner effective to enrich the cell population for stem cells; and

(c) exposing the enriched stem cell population[,] *ex vivo* to one or more antisense morpholino oligomers, having a substantially uncharged backbone and a base sequence directed to a target sequence spanning the translational start codon or an intron or exon junction site of an mRNA preferentially expressed in stem cells,

under conditions effective to (i) to increase the population of lineage committed progenitor cells and their progeny in the peripheral circulation of the subject, and/or (ii) effect a slowing or diminution of the growth of cells exhibiting a loss of growth control, or a reduction in the total number of such cells; and

(d) infusing the antisense oligomer-treated cell population into said subject.

12. (Amended) The method according to claim 10, wherein each of said one or more antisense oligomers have a length of about 12 to 25 bases.

13. (Amended) The method according to claim 10, wherein each of said one or more antisense oligomers [are] is characterized by[,]

(a) a backbone which is substantially uncharged;

(b) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;

(c) nuclease resistance; and

(d) the capability for active or facilitated transport into cells.

14. (Amended) The method according to claim 10, wherein said antisense morpholino oligomer [backbone has a structure selected from the group consisting of the structures presented in Figures 2 A-A through 2 E-E] comprises phosphorodiamidate intersubunit linkages, joining a morpholino nitrogen of one morpholino subunit to a 5'-exocyclic carbon of an adjacent morpholino subunit.

15. (Amended) The method according to claim 11, wherein each of said one or more antisense oligomers [have] has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12.

16. (Amended) The method according to claim 15, wherein [said antisense compound] each of said one or more antisense oligomers has the sequence presented as SEQ ID NO:1.

17. (Twice Amended) A composition comprising an antisense morpholino oligomer characterized by a backbone which is substantially uncharged, where said oligomer is directed to a sequence spanning the mRNA translational start codon of a gene preferentially expressed in stem cells.

18. (Twice Amended) The composition according to claim 17, wherein said antisense oligomer has a base sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:12 [is characterized by,

(a) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C;

(b) nuclease resistance; and

(c) the capability for active or facilitated transport into cells].

19. (Amended) [The composition according to claim 18] A composition comprising an antisense oligomer having a substantially uncharged backbone, wherein said antisense oligomer is characterized by

(a) the ability to hybridize with the complementary sequence of a target RNA with high affinity at a Tm greater than 50°C,

(b) nuclease resistance, and

(c) the capability for active or facilitated transport into cells;

and has the sequence presented as SEQ ID NO:1.

20. (Amended) [A pharmaceutical composition comprising the] The composition of claim 19, further comprising a pharmaceutical carrier.